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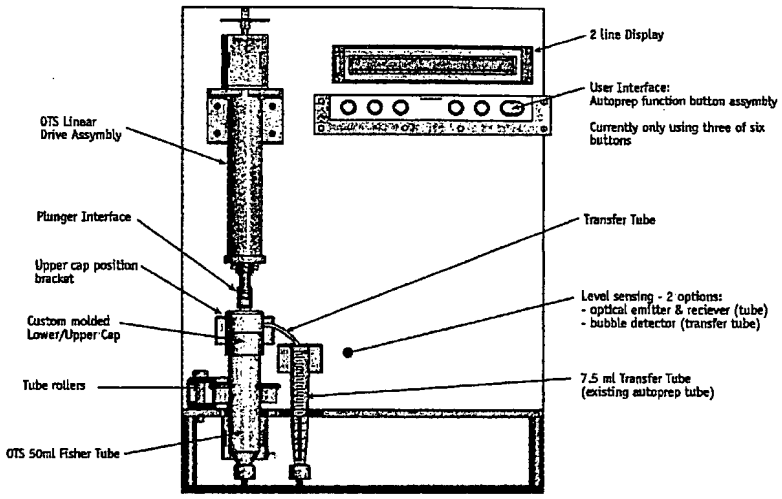
(54) Title: METHOD AND APPARATUS FOR PRE-ENRICHMENT AND RECOVERY OF CELLS FROM DENSIFIED WHOLE BLOOD



(57) Abstract: The present invention describes reagents, methods and apparatus for pre-processing of blood samples to obtain blood fractions containing substantially all target cells in reduced specimen volumes that are suitable for subsequent further enrichment. Sample processor systems are described for enriching cell populations having densities of less than approximately 1.09 g/ml from about 30 ml whole blood. Whole blood is combined with a cell compatible dense reagent to raise the density to about 1.09 g/ml. Centrifugation causes the target cells and most of the WBC in the buffy coat layer to accumulate at the top under a low-density buffer overlay. The reduced sample volumes are suitable for subsequent immunomagnetic or other enrichment methods prior to detection and enumeration. These processor systems, in conjunction with other enrichment procedures, facilitate detection of rare target cells, such as circulating tumor cells (CTC) that are useful markers in cancer diagnosis and monitoring of therapeutic response.

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Title: Method and Apparatus for Pre-Enrichment and Recovery of Cells from Densified Whole Blood.

Inventors: Steve Gross and Herman Rutner

Priority Information

This application claims priority under 35 USC §119(e) to US Provisional Application 60/520,614, filed 17 November 2003 and incorporated by reference herein.

Background

- Field of the Invention

The present invention relates to the field of centrifugation and fractionation of a blood sample. More specifically, using non-gradient conditions, the invention produces an enriched blood fraction in a reduced volume containing substantially all the target cells at the top of the sample, thereby enabling removal for subsequent further enrichment and analysis, as well as allowing safe containment and disposal of unwanted hazardous blood fractions.

- Background Art

Circulating tumor cells (CTC) in the blood of cancer patients, unlike the more homogeneous populations of cultured tumor cells, are known to have diverse densities depending on cell size, nuclear contents and overall integrity that generally fall in the range of 1.05 to 1.10 g/ml. Thus, rare target cells such as morphologically intact and viable CTC may be found both in the buffy coat layer, containing most of the white blood cells (WBC), and to a lesser extent in the red blood cell (RBC) layer after centrifugation. Trapping of the lighter WBC/CTC in the RBC layer, which is typically discarded during buffy coat isolation, is believed to occur due to the high RBC downward flux during conventional centrifugation of undiluted blood. Hence, a substantial and variable number of target cells may be found in the lower region, resulting in their exclusion from a buffy coat layer normally harvested for WBC/CTC. As a consequence, an unacceptably high loss of target cells may ensue.

Density gradient centrifugation is commonly used as a method of separating cells based on the different densities of cell types in a mixture. Cells can be separated by a single step into two compartments which contain cells that are either lighter or heavier than the specific density of the gradient material used. But a single density usually does not generally allow the cells of interest to be enriched to a significant level of purity, especially when the cells are present in a low number among other cell populations. A series of different density gradients or in combination with affinity chromatography, cell panning, cell sorting, or immunomagnetic enrichment are frequently used. Discontinuous density gradient centrifugation can be utilized using multiple layers of different gradient densities. This method forms bands of fractionated cells at their corresponding densities that can be collected by placing a pipette tip or similar aspirating device at the appropriate band.

Since cells can be fractionated on the basis of differences in their buoyant densities, density gradient methods are commonly used to fractionate cell populations in whole blood and other fluids. Such methods generally require dilution of whole blood with a buffer and careful overlaying the mixture with one or more gradient media of decreasing densities. After centrifugation, cells of different densities accumulate in the buffer layers of the corresponding density or at the gradient interfaces from which they can be carefully aspirated. Such techniques are well known in the art and described in great detail in several manuals or books (Biological Centrifugation, J.M. Graham, and D. Rickwood, eds, 1999).

Blood is "densified" blood when whole blood is spiked with a denser medium to increase the density of the plasma in whole blood. Densification is accomplished with any blood-compatible dense medium known in the art. The preferred densifier is iodixanol, such as OptiPrep (WO 00/47549, Nycomed Imaging SA), a non-ionic iodinated fluid used as an X-ray imaging agent and in density gradient separations. Iodixanol has highly desirable characteristics, e.g. high solubility in water (at least 60%), high density (1.32 g/ml) at 60%, low viscosity and osmolality (about 180 mosm) and low cytotoxicity, all of which make it ideally suited for preparing density

gradients. Salts of the structurally related diatrizoic acid also form aqueous solutions that have high densities, but are less desirable since they have a lower solubility and higher osmolality at comparable densities of iodixanol.

Iodixanol is commonly used in density gradient fractionations. In a standard non-gradient protocol, the density of the plasma in whole blood is increased to only about 1.077 g/ml. Consequently, this protocol floats only the lighter mononuclear cells to the top of this layer, but not the more dense polymorphonuclear leukocytes (PMN) or granulocytes, which remain at the interface or embedded in the RBC layer. Cells having a density of greater than 1.077 g/ml, which includes the more dense fraction of CTC, may be lost. Thus, potentially lower recoveries of CTC and other target cells may occur when using this conventional protocol. Disregarding these losses is intolerable when isolating rare target cells, including CTC, that may occur at frequencies of 1 to 2 CTC in 30 mL of whole blood.

Gradient protocols in the art use dense fluids to increase the density of leukocyte-rich blood fractions containing the target cells, but these enriched fractions are prepared in a preliminary gradient fractionation step of whole blood. For further fractionation, the enriched fraction is adjusted to a density of 1.10 g/ml with iodixanol and then overlaid sequentially with two other gradient media of decreasing densities: d=1.084 g/ml and d=1.068 g/ml. A small buffer overlay of much lower density, typically about d=1.02 g/ml, is also used to protect any accumulated cells from the damaging effect of the buffer/air interface. After centrifugation, the granulocytes collect at the top of the RBC layer and the lymphocytes and monocytes are separated in the overlaid gradients. Hence, this protocol requires pre-fractionation of whole blood, partitioning into three gradients and tedious sequential aspiration of several layers to isolate the target cell populations.

Other density gradient methods for buffy coat separations require diluting blood to reduce the density and viscosity (i.e. using a 1:1 dilution with a low-density buffer). After centrifugation, aspiration of a large volume of supernatant plasma is required before reaching the target cells in the buffy coat layer, located near the RBC

interface. Manual aspiration of the buffy coat layer requires good technique and patient skimming to ensure complete aspiration of the compact layer at the interface with the RBC layer. Hence, these manual procedures do not provide the reliability and reproducibility required for maximal and consistent recoveries of rare cells, especially in determining CTC for diagnosing disease. Multiple processing or gradient steps also lower the target cell recoveries, since overall yields of target cells are the product of the fractional recoveries at each step.

Besides manual aspiration methods, automated instrumentation has been described in the art for separating segregated blood samples after centrifugation by a variety of methods including fluid displacement by slideable pistons or plungers. Like manual methods, automated methods also depend on sequential removal of plasma or gradient layers to isolate the target cells located in one or more lower layers. These procedures are especially inadequate for rare cell detection as such displacement methods may incur absorption losses due to contact with the large surface areas of the plunger or piston, thus lowering the yields of the target cells. In addition, the plungers or pistons are usually re-used, requiring efficient decontamination to minimize the potential of cross-contamination between processed samples. Further, losses of target cells may occur due to trapping within the densely packed RBC layers of conventional gradient systems, especially under high centrifugal force conditions such as during centrifugation.

Automated instruments, used in gradient fractionation, provide a more consistent collection of segregated layers, but suffer from the same procedural problems seen in manual methods. The removal of multiple fractions can be done either manually or automated with commercially available products (i.e. Auto-Densi-Flow ® Density Gradient Fractionator, LabConco Corp., Kansas City, MO). This automated process mimics essentially the manual aspiration process using a pipette by lowering a tubular probe to aspirate sequential fractions at a controllable rate. But in buffy coat aspirations, automation may actually be less efficient, since the probe is lowered in a horizontally fixed position, whereas in a manual method a pipette tip can be swept back and forth in a horizontal plane to achieve more efficient aspiration. Thus, there

is a need to develop an automated instrument that will efficiently and consistently remove segregated layers containing target cells.

U.S. patent 4,003,834 describes a method and apparatus for separating the segregated components of a centrifuged blood sample. U.S. patent 5,645,715 further eludes upon this concept. In this patent, the trumpet shape is designed to enhance laminar flow of the sample while collecting. The large surface area of the trumpet is in direct contact with the target cells in the sample, increasing the risk of cell loss. Further, the reusable piston increases the risk of sample-to-sample cross contamination and requires time-consuming decontamination.

US patent 4,346,608 discloses a free-floating piston or plunger device having a concave shaped bottom to minimize turbulence during aspiration. Here, again, the re-usable plunger fully contacts the aspirated fluid, and thus has the same limitations with respect to potential cell losses. Further, the pulsating mode of the peristaltic pump is a potential source of damage to fragile CTC.

Application WO 03028844 describes the isolation of blood components from centrifugally segregated samples by means of a mechanically driven fractionator assembly.

The reproducibility and yield by manual aspiration methods with Pasteur pipets and gradient fractionation methods, utilizing stepwise increases in the gravity vector force (i.e. ramping), gave substantially smaller recovery amounts of target cells than needed for rare cell detection, making these manual aspiration methods unsatisfactory for use in diagnosis, prognosis, or any clinical analysis. For example, OncoQuick™ tubes sold by Greiner BioOne, Longwood, FL, along with issued patents US 5,474,687, US 5,663,051, US 5,789,148, and US 5,840,502, use a gradient fluid of about 1.069 g/ml under a frit plate or constrictions to segregate the centrifuged layers thereby permitting facile decanting of the separated buffy coat layer from the RBC. The upper decanted layer typically contains the plasma and is diluted by a portion of the density gradient material. The presence of a full plasma

layer makes these separation systems unsuitable for rare cell enrichment processes, primarily because of the removal of a major portion of the plasma.

Accordingly, an unmet need exists for simple, quick, and reproducible system of separation, either manual or automated, that provides nearly complete recoveries of WBC and rare target cells.

Summary of the Invention

The present invention provides methods and apparatus for non-selective pre-enrichment of cell populations having densities of less than approximately 1.09 g/ml. It consists, in part, of a non-gradient centrifugation method that uses a single homogenous layer for centrifugal enrichment of cells including target cells in blood, and a disposable sample tube as part of an apparatus that is configured to ensure virtually complete recovery of all target cells along with other non-target cells, located near or at the surface of the densified blood layer.

Accordingly after addition of the appropriate amount of densifier and subsequent centrifugation, nearly all cells of densities lower than approximately 1.09 g/ml are located in the uppermost portion. These cells can be removed manually or with an automated aspiration device for further selective magnetic enrichment prior to any analysis such as imaging and/or enumeration. Nearly all WBC populations having densities of less than approximately 1.09 g/ml (except for a small fraction of granulocytes with densities above 1.09 g/ml) and substantially all intact rare target cells, including CTC, are recovered in yields approaching 95%. These high recoveries may be due, in part, to the expanded RBC layer in the densified blood after centrifugation in which about half the RBC are loosely pelleted, minimizing trapping of CTC and WBC during centrifugation.

A further embodiment provides an improved system for ensuring consistent recoveries of enriched cell fractions located near the upper fraction of a densified blood mixture. These recoveries are far higher than achievable with conventional gradient separations such as with OncoQuick™ tubes. The method of the present

invention thus improves the recovery of rare cells using conventional gradient fractionation by eliminating the need for blood dilution, use of two or more gradient layers, and aspirating multiple fractions of a potentially hazardous specimen, particularly in collecting a target cell population from a large sample volume (e.g. 20-30 ml blood).

Critical components of the system include:

(1) An automated system for rapid and efficient preparation of large numbers of blood samples to be effectively used in a clinical laboratory.

(2) Safe collection of an enriched blood fraction and disposal of contaminated instrument components.

(3) A slideably sealed piston or plunger, automatically or manually driven, generating an enclosed air space to force fluid into a collection reservoir.

(4) A plunger head, having an optimally shaped collection duct on the fluid surface, optimally mounted along the plunger surface.

(5) An air space between the plunger surface and the fluid surface imparting a uniform pressure on the fluid surface when the plunger is driven which forces the desired amount of fluid into a collection duct.

(6) A optimum ratio of cross-sectional diameter between the collection duct and plunger, ranging from about 1:10 to about 1:1.5.

(7) Virtually complete collection or a high percentage of the WBC and rare target cells having densities of less than 1.09 g/ml, but excluding cells with substantial morphological damage (i.e. damaged membranes) or having densities of greater than 1.09 g/ml.

Brief Description of the Drawings

Figure 1: Manual preparation of a blood sample for pre-enrichment processing. About 5 ml, or a function of the hematocrit, of iodixanol are added to 30 ml of whole blood in a 50 ml centrifuge tube, gently mixed (Step 1), and overlaid with appropriate cell protective buffer (Step 2). After centrifugation, the white blood cells (WBC) and the target cells (TC) along with some RBC are partition in the upper portion of the

sample volume (Step 3). Plasma and the bulk of the red blood cells (RBC) constitute the remaining lower fraction. The standard tube cap is then removed and replaced with the large volume sample processor (LVSP) plunger cap of the present invention (Step 4).

Figure 2: Comparison showing the partitioning of normal whole blood with/without iodixanol, followed by centrifugation. Also shown are the 50 ml conical centrifuge tube and the iodixanol -treated sample overlaid with about 500 ul buffer.

Figure 3: Processor apparatus with 50 ml conical centrifuge tube containing the sample and the collection vial. After the user loads the centrifuged sample tube into the sample processor apparatus, the plunger device is attached to the centrifuge tube, and the transfer tubing is attached to the sample collection vial.

Figure 4: Removal of separated sample after aspiration of the buffy coat. After displacing the appropriate volume of up to about 7.5 ml or ¼ of the original blood volume), the plunger is returned to the initial starting position and any residual sample is drawn back into the centrifuge tube. The plunger or LVSP cap on the 50 ml sample tube is then rotated on its longitudinal axis to retract the contaminated transfer tubing back into the cap for safe containment and disposal of the entire plunger-tubing device along with all of the remaining blood in the tube into a biohazard receptacle.

Figure 5: Diagram of the 50 ml sample tube with LVSP cap attached. (A) Sample tube with sealed cap prior to use. (B) Sample tube with cap removed and transfer tubing unwrapped. (C) Sample tube re-capped with transfer tubing retracted through an opening (mouse hole) in cap.

Figure 6: Detailed representation of a cross-sectional view of the snap cap on the sample tube.

Figure 7: Detailed representation of a cross-section view of the snap cap integrating a trumpet shape into the fluid surface of the plunger head to form a conical collection duct.

Figure 8: Cross sectional view of the plunger motion. (A) The plunger is in the home position with the LVSP cap/conical tube attached. (B) The plunger is in the extended position, prior to retracting to the home position.

Figure 9: Diagramatic representation of instrument components of the present device.

Figure 10: Detailed representation of sample tube attached to a processor system with bracket support.

Figure 11: Detailed representation of the plunger-reservoir device, incorporating a reservoir within the plunger.

Figure 12: Percent WBC recovered in buffy coat using LVSP with ¼ inch collection duct off-center, on-center, or flush on-center with respect to the sample surface of the plunger head.

Detailed Description of the Invention

- General definitions

Unless otherwise indicated, terms of general usage throughout the present specifications have customary meanings.

The term "rare cells" as used herein refers to a variety of cells, microorganisms, bacteria, and the like that are either cells not present in normal samples or are normally present at several orders of magnitude lower than the majority of the cells in a normal sample. In one embodiment of the present invention, the rare cells are exemplified as circulating cancer cells, virally, infected cells, or fetal cells in maternal circulation that can be efficiently isolated from non-rare cells and other bioentities,

using the methods and apparatus of the present invention in conjunction with previously described technology (US 6,365,362).

The term "densified" or "densification" refers to increasing the density of the fluid portion in a specimen, e.g. whole blood specimen or bodily fluids, by adding a cell compatible medium resulting in a mixture of increased density, typically with a target density of about 1.09 g/ml.

The term "buffy coat", as used herein, refers to the WBC layer formed in whole blood or densified whole blood after partitioning a blood sample by centrifugation. The "buffy coat" consists mainly of WBC with some granulocytes excluded in whole blood and containing substantially all the rare target cells that have densities similar to WBC.

The term "Large Volume Sample Processor" (LVSP) refers to the collection apparatus developed to selectively collect the upper layer of a densified specimen, containing target entities or, as an alternative, to separate and collect sequential layers after a conventional gradient centrifugation and partitioning.

The term "enrichment" refers to an increase in the concentration of desired rare cells in a smaller fluid sample during blood pre-processing, or it may refer to an increase in the ratio of target to non-target cells during subsequent magnetic processing.

The term "toxicity" herein refers to a diminution in cell viability or biological function that is measurable in a cell population.

The term "non-gradient centrifugation" refers to centrifugation of a densified specimen mixture resulting in a single partition between the buffy coat at the upper region and the red cell layer below, e.g. the non-gradient partitioning of whole blood results in a buffy coat layer, along the upper portion of the fluid sample, that contains white cells and target cells.

The term "floating" cells refer to cells located along the surface of the sample after partitioning the cells by non-gradient centrifugation.

- Whole blood analysis

Whole blood is a highly complex mixture of cellular and soluble components in which RBC and albumin predominate. While the composition of normal blood is quite uniform with respect to the major components, many of the lesser components are present in variable amounts, particularly in diverse disease states. Specimen from patients may contain cellular pathogens including circulating tumor cells (CTC) that are shed from primary, secondary or metastatic tumor sites. Such cells, classified as rare cells, may be prevalent in the blood as low as 1 to 2 CTC per 30 ml of sample from a patient with early stage cancer. This is exceptionally low when compared to about 5 million leukocytes per milliliter or 10 billion erythrocytes or red blood cells (RBC) per milliliter.

Substantial enrichment of CTC at high recoveries is obviously essential in any analytical method of CTC detection. The development of diagnostic instrumentation used in the enrichment, isolation, detection, and enumeration of rare cells has made possible the clinical application of specific tests for the presence of CTC at various disease stages and their use in cancer diagnostics and prognostics. The pre-enrichment method of CTC of the present invention further improves upon these cancer tests by removing a substantial portion of the non-target components in whole blood, which frequently interfere with the selective and efficient isolation of CTC.

- Densification of whole blood

Using a densified whole blood specimen, the present invention ensures nearly complete partitioning of target cells near the surface portion of the mixture, containing cells and cellular components having densities up to about 1.09 g/ml. This would include CTC, most WBC and a small amount of RBC.

Increasing the density of plasma in the blood from the normal density of about 1.025 g/ml to about 1.09 g/ml expands the RBC layer from typically 30% to 55% up to about 95% of the whole blood volume. The small plasma layer near the surface contains some lighter RBC populations mixed with the buffy coat cells and the desired rare or target cells. Hence with the increased plasma density, there is no need to aspirate the plasma layer prior to the removal of the target cells from the buffy coat layer as required for gradient separation. Further, an overlay of the blood mixture prior to centrifugation with a small volume of isotonic and non-cytotoxic fluid containing polyvinylpyrrolidone or protein in a density range of about 1.01 to 1.05 g/ml (such as, but not limited to, PBS buffer with 0.5% BSA at a density 1.025 g/ml) minimizes cell lose from known surface tension-induced damage at the air/liquid interface and other associated factors. The addition of albumin in the overlay reduces the surface adhesion of cells to the wall of the centrifuge tube.

The present invention, in part, embodies a system with the following specifications for a large volume sample preprocessing (LVSP) device, developed for enrichment and minimal loss of target cells: (a) Segregation of the target cells in the sample in a relatively small volume, approximately one fourth of the original blood volume; (b) Localizing the target cells near the surface of the densified blood sample for easier removal, preferably under a small layer of protective buffer fluid to minimize losses of target cells that would occur at the air-fluid interface or on the walls of the centrifuge tube; (c) Recovering WBC at greater than about 90%, to reflect comparable recoveries of target cells or CTC; (d) Reducing contamination to no more than 10% of the RBC from the original blood sample, a level found tolerable in subsequent magnetic enrichment processes. Contamination from RBC can generally be avoided by densifying the plasma to about 1.085 g/ml; (e) Reducing contamination from the original plasma volume in the enriched blood fraction to less than about 25% of the original plasma volume in order to minimize potential protein interferences in any subsequent magnetic enrichment process; and (f) Ensuring compatibility with blood specimens containing rare cell stabilizing compositions such as, but not limited to, those described in PCT/US02/26861 and PCT/US02/26867.

Densification typically involves mixing whole blood with a blood-compatible dense fluid as exemplified, but not limited to, 60% iodixanol (d = 1.32 g/ml; 180 mosm), to a target density of about 1.09 g/ml prior to centrifugation. Typically, densification is followed by centrifugation, either with or without ramping to full speed in order to complete CTC partitioning. While centrifugation is the preferred method for partitioning, any procedure known in the art to partition whole blood is appropriate (i.e. partitioning by gravity flotation). One option, not commonly practiced, is to store the densified blood tube flat overnight after treatment with a densifying agent, either with or without the addition of the blood stabilizers/fixatives. Densification and centrifugation can be done in any blood containment vessel capable of centrifugation. 15 ml or 50 ml conical centrifuge tubes are preferred, primarily for disposability and cost. Prior to centrifugation, the mixture is overlaid with a small volume of protein containing buffer (d=1.025 g/ml) before centrifuging for 20 min at 800 xg to 1500 xg at room temperature. Optionally, ramping or stepwise increases in the centrifugal force over a longer centrifugation time may further decrease trapping of target cells and increase recoveries. Any appropriately compatible densification material, added in a suitably tested proportion can be used to provide a desired density (i.e. 1.09 g/ml) while maintaining the osmolality within a range that does not alter the densities of the desired cell or cell components nor cause destruction of the same targets. The target cell or components are then collected in the first partitioned phase by removing this small volume from the initial whole blood. The partially enriched fraction is now suitable for further processing such as, for example, immunomagnetic enrichment or it can be directly used in an appropriate cell analysis system such as, for example, by flow cytometry. The partial enrichment method of this invention thus recovers essentially all rare target cells along with a limited number of non-target cells such as WBC and RBC which, quite unexpectedly, were found to reduce nonspecific binding in the subsequent immunomagnetic separation step. Such impure fractions provided an unexpected advantage over unprocessed whole blood or plasma-depleted blood in the subsequent highly selective enrichment steps involving magnetic separation and CTC detection.

In one mode, accurate densification is achieved by calculating the required amount of dense fluid based on the measured hematocrit (HCT) of the blood sample in order to increase the density of the plasma portion from a normal density of 1.025 g/ml to about 1.09 g/ml. Alternatively, densification can be done based only on blood volume. However, if a fixed percentage of the dense fluid is added to blood (i.e. 15% by volume of iodixanol) the resultant density of the plasma fraction will vary, depending on the hematocrit or percentage of RBC in the whole blood sample. Accordingly if a consistent density is required, a preferred alternative is to add the appropriate amount of densifying agent based on the actual HCT value of the blood specimen (i.e. typically ranging from 30 to about 50%). The final density of the mixture also affects the RBC distribution in the plasma and the amount of RBC aspirated in the top portion containing the target cells. Thus at a high HCT, the density of the densified plasma is higher than 1.09 g/ml, and the top fraction may contain as much as 25% of the total RBC in the blood sample. The converse is true at low HCT, which increases the volume of the plasma layer and reduces the RBC contamination to less than about 5%. The larger and less dense plasma layer thus contains only the lighter RBC fraction mixed with the buffy coat and target cells near the top interface. By increasing the plasma density, there is essentially no plasma layer to aspirate prior to removal of the buffy coat.

The densified blood is then overlaid with a small volume (less than 5%) of a protein-containing buffer, such as PBS+0.5% albumin (d=1.005 g/ml), as mentioned above, to protect the CTC from any damage induced by surface tension at the air/liquid interface. More importantly, the presence of protein is a factor in reducing losses of WBC and presumably also of target cells due to adhesion to the vessel surface during displacement of the top fraction.

A more accurate separation of WBC and CTC from RBC, also embodied in the present invention, is accomplished by changing the plasma density to 1.085 g/ml, as assessed by the HCT. Using this density, most of the WBC and CTC are located near the top of the plasma layer, but essentially none of the RBC are aspirated with top layer.

Whole blood stabilized with cell preservatives or fixatives, such as the CytoChex™ System and the TRANSFIX™ System and that as described in WO 03018757, can be similarly densified and processed. These known stabilizers allow storage of whole blood specimen for several days without significant losses of rare target cells. Premixed blood stabilizer is premixed with a densifing agent, such as the Optiprep additive. A typical ratio of cell preservative agent to Optiprep agent is about 0.3 to 1.2 ml, respectively, in a 10 ml blood tube.

Thus, a further embodiment of the present invention incorporates, in part, whole blood densification on blood samples that are pretreated with a cell preservative or fixative, either added before densification or concurrently as a preservative-densifier cocktail. A densifier such as, but not limited to, iodixanol can be premixed with the cell preservative in a 10 ml evacuated blood draw tube, in a preferred ratio of about 0.3 to 1.2 ml, respectively, or a total volume of about 1.5 ml. The osmolality of this mixture is thus reduced to about 500 mosm from the 1750 mosm value, using the preservative in WO 03018757. Consequently, the mixture reduces the hyperosmotic shock on sensitive cells during blood draw (note: the EDTA in the BD Vacutainer® tubes is about 1100 mosm and blood diluted with the Cyto-Chex™ cell stabilizer has an osmolality of about 360 mosm). The combination of densifier and cell preservative agent formulation (or other appropriate stabilizer) in a prefilled evacuated blood collection tube was found to be an effective, safe, and easy-to-use option compared to conventional blood tubes that would require subsequent addition of the densifier to the blood or blood draw tube.

Further, the combination of densifier and fixative provides a buoyant density medium during overnight storage of blood samples in centrifuge tubes. Upright storage keeps the WBC and CTC suspended at or near the top of the tube, thereby further decreasing possible trapping during subsequent centrifugation.

As previously stated, the amount of densifier needed for achieving a density of 1.09 g/ml in densified blood plasma is dependent upon the hematocrit and blood volume

used. Consequently, the hematocrit must be determined to adjust the density. Without adjustment, any differences in hematocrit will alter the distribution of RBC, specifically the percent of RBC in the buffy coat containing the WBC and CTC. The hematocrit is rapidly determined in assessing the routine blood profile (such as with the Sysmex® analyzer or any analyzers used in most clinical labs). An alternative, when RBC distribution is not critical, is to add a fixed amount of densifier to the blood based solely on the measured volume thereby accepting any variation in plasma densities and RBC contamination, similar to the procedure described above.

Densification provides several advantages in the enrichment process. Expanding the RBC layer has the advantage of less WBC and/or CTC trapping in the normally rapidly descending RBC during centrifugation, particularly in the presence of high gravity vectors, which ultimately forms a densely packed RBC layer from which the lighter cells cannot escape. The resultant WBC losses from this trapping effect can approach 50% depending on the ramping rate of the centrifuge, gravity vector, the volume of blood, and the dimensions of the centrifuge tube. A further advantage of the present invention is that the ramping rate is less critical than in conventional density gradient centrifugations due to the expanded RBC layer in densified blood resulting in less WBC and/or CTC trapping.

It is understood that the centrifugation of densified blood (with or without pretreatment with any cell preservative) can be conducted inside other devices commonly used in the art (i.e. a centrifuge tube, blood bag or sample fluid vessel capable of centrifugation). For each type of vessel, plasma aspiration is not needed as all cells of densities less than 1.09 g/ml, including most WBC and CTC are collected from the upper partitioned layered, possibly mixed with some plasma and RBC. The RBC contamination can be further reduced with the addition of conventional RBC aggregating agents, like dextran, hexadimethrine, or polyethyleneimine. The enriched top layer, containing the protective overlay and buffy coat components, can thus be reduced to about 10% of the initial blood volume.

Densification is not appropriate to positively select fragments or dead cells. As in any gradient system, perforated or dead cells of higher densities (>1.09) or cell fragments of lower densities, located outside the buffy coat layer, would be lost. Hence, for positive selection of the full range of intact, damaged or fragmented CTC the appropriate method is capture from whole blood or preferably washed plasma depleted blood, as is currently described in immunomagnetic capture protocols for rare target cells (US 6,365,362 and 20020172987).

- Sample processor system

Finally, centrifugation of the densified blood provides cell population partitioning allowing rapid and easy automation. Facile aspiration of the top portion containing the target cells with automated gradient separators provides a clinically viable system for rapidly processing large numbers of blood samples. One embodiment of the present invention is to integrate blood densification with a collection device in a system that allows acquisition of a pre-enriched sample suitable for analysis in a clinical setting. The system allows effective and efficient removal of the top portion of the centrifuged blood mixture in a safe and reliable format. Further, the system provides for the reduction of 20 to 30 ml of collected whole blood to less than about 8 ml.

Thus, the large volume sample processor (LVSP) is a rapid, safe, and low cost system to augment cell enrichment systems for use on analytical cell platforms, particularly in clinical diagnostics. The LVSP contains a 50 ml conical tube and holder, a custom molded cap which comprises a plunger, tubing and safety-related containment features, and instrumentation to automate the required steps for processing a sample.

As shown in Figure 1, the sample is manually prepared with the addition of a densifying agent such as, but not limited to, iodixanol reagent (5 ml; Step 1) followed by gently inverting of the sample mixture and the layering of an appropriate buffer such as, but not limited to, PBS-0.5% albumin (Step 2). The mixture is centrifuged (Step 3) and recapped with a LVSP cap prior to processing (Step 4). As shown in

the representation of in Figure 2, the buffy coat partitions in the upper region of the densified blood. Compared with the partition profile of whole blood alone, the buffy coat is more accessible from the top which requires no plasma removal.

As shown in Figure 3, the sample is placed into the instrument for processing. The user uncoils the collection tubing and inserts the distal end into a retaining clip over the receiving vial. As the plunger is systematically lowered into the 50 ml conical centrifuge tube, the buffy coat is forced into the collection port and approximately 8 ml or less is collected.

Critical for clinical applications, the processor system removes densified blood with minimal user involvement (see Figure 4). As the plunger is withdrawn to the starting position, the unwanted fluid remaining in the collection tube is drawn back into the 50 ml conical centrifuge tube, minimizing the risk of spillage of a biohazard substance. When the plunger reaches the initial position, it disengages from the machine allowing for rapid and safe removal of the blood tube assembly after drawing the collection tubing back into the cap by rotating the cap. Also important for safety, all components in direct contact with blood samples are disposable. This includes the sample conical tube, its cap, and the plunger cap with integral transfer tubing. As shown in Figure 5, a typical conical tube/LVSP cap configuration prior to processing is formed by removing the standard conical tube cap after centrifugation, and replacing with the sealed LVSP cap. The inner seal on the cap is broken and the transfer tubing is pulled out. After blood processing, the transfer tubing is retracted and coiled on the shaft inside the inner portion of the cap by rotating the cap. The conical blood tube and plunger assembly are then safely discarded as biowaste. Figure 6 shows the detailed layout of the LVSP cap, attached to the conical tube in a cross sectional view.

Quite surprisingly and inexplicably, we found that the plunger design and apparatus of the present invention operates optimally and very effectively within a very low ratio range of collection duct to sample tube diameter. Significantly higher WBC and

target cells were recovered at ratios lower than 1:10 when aspirating the top portion in the densified blood.

Using a very low ratio of collection duct to sample tube diameters, plunger head, coupled to a mechanical drive device, allows efficient removal of the sample's top portion with minimal losses of target cells. A typical collection duct will have an inner tubing diameter of 0.25 inches for both the 50 ml and 15 ml conical centrifuge tubes. The opening for the collection duct ranges from about 1/8 inch to about ½ inch diameter. Further, the collection duct is located on the sample face of the plunger (Figure 7), either in the center or on one side of the surface.

In one embodiment, the collection duct has a conical shape that tapers from about 1/4 inch at the fluid inlet to mate with the 1/8 inch diameter of the collection tubing (see Figure 7) that is optimal for efficient collection of about 8 ml of the upper portion of the sample in the sample tube. Hence, separation of the top or upper portion of the densified blood sample, as described, allows collection of 90-95% of the target cells in about 20% of the original blood volume.

For a typical aspiration, the sample conical centrifuge tube with the LVSP cap is positioned in the processor apparatus with the plunger in the home position (Figure 8). The plunger moves at a predefined rate towards the sample. The descent rate of the plunger establishes the aspiration rate. While not limiting in the present invention, the typical decent rate of approximately 6.35 mm/minute translates to an approximate aspiration rate of 3 ml/minute in a 50 ml conical tube. The typical aspiration distance for collecting the top portion is approximately 12 to 15 mm or about 7.5 ml of a 35 ml densified blood sample.

To ensure a standardized aspiration and consistent volume, a fluid sensor is used to detect when the sample flows from the 50 ml tube into the smaller receiver tube. Thus, the instrument utilizes sensors to determine the amount of fluid aspirated. The fluid sensors also serve as an error detector to identify when fluid has begun to flow from the source vessel to the target vessel(s) and to sense when re-aspirating the

residual fluid in the tubing has been accomplished. A complete diagramatic view of the device is shown in figure 9.

Prior methodologies report only approximately 80-85% WBC recovery. The recoveries in the present invention result in high reproducibility and recoveries of 90-95% of target cells with diameter ratios of 1:5 to 1:8. With these significantly lower ratios, the present invention clearly provides an optimum sensitivity and cell selectivity for pre-enrichment of densified whole blood, not observed in prior reported methods. Figure 10 depicts the sample tube as attached to the apparatus and details the typical plunger motion with the sample tube attached to the processor apparatus. This configuration allows quick and safe processing of densified blood samples and is expected to function equally well for conventional gradient blood samples.

A further embodiment of the present invention consists of a compact integral and inexpensive assembly in which the plunger is in direct communication with the receiving reservoir thus eliminating the need for the transfer tubing and the LSVP cap. One of several configurations of this modification is shown in Figure 11 in a plunger-reservoir device, whereby a plastic reservoir, incorporated into the plunger component, forms a seal when the assembly is slideably inserted into the outer tube containing the centrifuged sample. An airtight o-ring or flexible plastic rim seals and thus creates an air space between the fluid surface and the plunger head. As the plunger-reservoir is further inserted in a stepwise manner, either manually or mechanically, air is displaced through the collection duct and into the reservoir until the collection duct contacts the fluid surface and prevents further escape of air. On further stepwise downward movement of the assembly, the resultant air pocket transmits uniform pressure from the descending piston to the fluid surface. The air pressure causes sequential surface layers to flow laterally towards the fluid duct in direct communication with the reservoir. In conventional gradient separations this device allows incremental removal of adjacent fluid layers (e.g. by pipetting) from the receiver tube. However, for use with densified blood, a fixed fraction of the original blood volume (e.g. 20%) is collected in the calibrated reservoir. The collected

fraction is removed by simple pipetting from the reservoir, with or without rinsing of the reservoir, to effect complete transfer of the fluid and target cells. A cap is placed on the reservoir to fully seal the enclosed residual blood prior to transporting the assembly to a biohazard container for safe disposal with minimal risk to personnel.

Besides diameter ratios (preferably 1:2 to 1:10), parameters such as flow rates controlling air pressure, depth of tip immersion, location of inlet (center or off-center), inlet shape (conical or straight) were found to affect the fractionation efficiencies or cell recoveries. But the main factors are the rate of descent of the plunger, tubing diameter and length, and the hydrostatic head of the fluid in the tubing ranging from positive (positive pressure in the air pocket) to negative (negative pressure or vacuum in the air pocket) depending on the relative positions of the tubing outlet above or below the tubing inlet, respectively.

Efficient operation of either plunger configuration requires a captured air pocket between the plunger and the surface of the sample fluid for efficient cell separation and high recoveries of cell populations. With the reservoir in communication with the sample, fluid is displaced upward and into the receiver tube, preferably the conical centrifuge tube used for subsequent enrichment, located adjacent to the plunger-tube assembly. The plunger-reservoir combination is a single-use device that is ideally suited for, but not limited to, rare cell enrichment from densified whole blood since it eliminates common sources of cell losses and cross contamination.

Accordingly after centrifugation, the plunger-reservoir assembly is inserted into the sample tube, and then either manually or mechanically moved downward until the desired volume of densified blood containing the target cells has been collected in the reservoir. The collected fraction is aspirated and transferred from the reservoir to another tube for further processing. The reservoir can be rinsed to obtain a more efficient transfer of the fluid with target cells. When used for CTC analysis with densified blood (d=1.09), the top fraction is collected. The RBC contamination present in this fraction could be avoided by incorporating a small amount of an RBC aggregation reagent such as, but not limited to, hexadimethrine. Alternatively,

multiple sequential fractions as low as 0.1 ml could be aspirated in a stepwise mode to achieve the highly efficient fractionations found in conventional gradient separations.

The present invention has applications in biochemical, clinical and diagnostic fields. For example, LVSP, used on whole blood from cancer patients, provides a useful pre-enrichment process when combined with immunomagnetic capture or any other known enrichment method for the separation of rare target cells. With immunomagnetic capture as described in US 6,365,362, the enriched CTC fraction are less affected by the known interferences due to plasma factors and RBC. The pre-enriched sample enables more efficient labeling and capture of target cells with antibodies linked to ferrofluid such as antibodies against epithelial-derived cell adhesion molecule or EpCAM. Pre-enrichment further reduces the amount of ferrofluid needed and facilitates removal of excess ferrofluid that must be removed before analysis. Pre-enrichment also reduces the potential interference from plasma components, which commonly occurs with blood from patients and even healthy individuals. Also, densification can be used as part of any cell separation procedure (i.e. in general buffy coat separations) where a high yield of WBC with minimal RBC contamination is desired. Densification provides faster and more efficient collection of target cells when used in conjunction with the sample processor system since only the top layer is aspirated.

Example 1: Collection Rate for the Processor System

In order to determine the optimum removal rate of the processor system, the WBC count in both the aspirated and residual portion was determine. Variable removal rates of the densified blood sample were used in a modified system by adjusting the speed at which the plunger displaced the sample volume.

Accordingly, blood drawn into tubes, used with WO 03018757 cell preservative, were stored at room temperature overnight. The sample was pooled and 30 ml transferred to a 50 ml centrifuge tube. Five ml iodixanol (OptiPrep™ System, Sigma) was added to the blood and the blood/ iodixanol mixture was mixed several times by inversion. After a five minute waiting period, 500 µl of PBS with 0.5% BSA was

gently overlaid on top of the blood. The blood was then centrifuged at 800 xg for 20 minutes without ramping. The overnight exposure of blood to cell preservative improved the separation of the CTC/WBC fraction from whole blood.

The sample was then placed on an automated processor system with a fixed 1/16 inch inner diameter collection duct and tube, designed to allow variable removal rates of a densified blood sample. By adjusting the speed at which the plunger displaced the sample volume, the buffy coat could be collected in a rate dependent fashion. A controller on the apparatus was designed to drive the plunger vertically into a 50 cc conical centrifuge tube. The collection rates tested were 0.125 inches/min, 0.25 inches/min, 0.325 inches/min, 0.43 inches/min, 0.65 inches/min, 1.25 inches/min, and 2.5 inches/min. As the plunger travels longitudinally down the barrel of the 50 ml centrifuge tube, the buffy coat as well as the layers immediately underneath were collected via a 1/16 inch collection duct and transferred via 1/16 I.D. tubing to a 15 ml centrifuge tube adjacent to the 50 ml tube. After a total of 8 ml was collected, the plunger was halted and reversed to its starting position. The plunger was then removed from the 50 ml centrifuge tube. Both the buffy coat material in the 15 ml centrifuge tube and the buffy coat-depleted blood in the 50 ml centrifuge tube were well mixed. 200 µl from each were removed and a complete blood count was performed on a Sysmex™ Hematology Analyzer (Sysmex America Inc., Mundelein, IL)).

A total white blood cell count (WBC) was then determined for both fractions. The WBC was expressed as a percent WBC recovery in the buffy coat and determined by the formula in equation 1:

Equation 1:

% WBC = buffy coat fxn/(buffy coat-depleted fxn + buffy coat fxn) x 100

where % WBC is the percent WBC, buffy coat fxn is the number of cells present in the buffy coat fraction, and buffy coat-depleted fxn is the number of cells present in the buffy coat-depleted fraction.

The data of this preliminary study shows a maximum WBC recovery of 87% +/- 1.8 with a plateau in the recovery at a rate at 0.25 inches/min.

Example 2: Cross-sectional diameter for the Processor System

In order to determine the optimum collection duct and tubing diameter for white blood cell recovery, several plungers were constructed which contained collection ducts in a variety of inner diameters. Inner tubing diameters were as follows; 1/32inch, 1/16inch, 1/8inch, 3/16inch, and 1/4inch. The corresponding ratios of the inner diameter collection duct to centrifuge tube are as follows; 1:34, 1:17, 1:8.5, 1:5.7, and 1:4.25, respectively. For the large I.D. tubing, considerable amount of buffy coat did not travel effectively to the collection container. Because this problem did not occur with collection tubing of 1/8 inch or less inner diameter, a plunger head was designed with a conical collection duct extending from the plunger surface. The collection duct thus had a tapered shape with a ¼ inch inner diameter, on the fluid surface, and tapering to smaller 1/16 inch diameter tube connected to the collection tube. This plunger-tubing configuration provided the benefits of both optimal duct and tubing diameters and enabled the highest cell recoveries.

In a typical blood separation protocol, whole blood is drawn into collection tubes (WO 03018757) and stored at room temperature overnight. The sample is pooled to 30ml and transferred to a 50ml centrifuge tube. Five ml of iodixanol is added to the blood and the blood/iodixanol mixture mixed several times thoroughly by inversion. The blood is then allowed to stand for 5 minutes. During this five minute waiting period, 500 µl of PBS with 0.5% BSA is gently overlaid on top of the blood. The blood is then centrifuged at 800xg for 20 minutes.

The sample is then placed on the processor system of the present invention and the plunger attached. The plunger rate is then set at 0.25 inches/minute and 8 ml of buffy coat and associated material are collected. Both the buffy coat fraction material in the 15 ml centrifuge tube and the buffy coat-depleted blood fraction in the 50 ml centrifuge tube are well mixed. 200 µl from each were removed and a complete blood count performed on a Sysmex Hematology Analyzer. A total white

blood cell count (WBC) is determined for both fractions. The WBC from this determination is then expressed as the percent WBC recovery.

The following results for WBC recoveries were observed for plungers with collection ducts possessing inner diameters of 1/32 inch, 1/16 inch, 1/8 inch, and ¼ inch (Table 1).

Table 1.

Collection duct diameter	Ratio of Collection duct to sample tube	Mean WBC Recovery
1/32 inch (1:34)	78.5 +/- 4.5%	79%
1/16 inch (1:17)	87.1% +/- 1.8	87%
1/8 inch (1:8.5)	89.3% +/- 2.2	90%
¼ inch (1:4.25)	90.4% +/- 2.9	91%

The results show that the mean recovery rates increased as the collection duct inner diameter, as well the ratio of collection duct inner diameter to the centrifuge tube inner diameter increased.

Example 3: Dependence upon collection duct position

The relative orientation of the collection duct along the plunger head surface was compared to determine optimum WBC recovery. In order to assess the significance of the relative position of the collection duct with respect to the plunger head surface, WBC recovery using LVSP was compared between two plunger head positions, each having a ¼ inch collection duct diameter, either in an off-center or on-center orientation. Further, the on-center orientation was modified in another design to be flush with the plunger's sample face (see Figure 7).

Results of this assay are shown in Figure 12. The WBC's recovered on LVSP with the three designs are expressed as the percent of WBC's recovered in the buffy coat. All three designs had greater than 90% WBC recovery. The flush design resulted in the highest percent recovery.

Example 4: Effect of the buffer overlay

After treatment with iodixanol and centrifugation, the buffy coat rises to the surface of the blood sample, directly contacting the liquid/air interface. As protection against exposure of the buffy coat to the liquid/air interface, a 500 µl buffer layer of phosphate buffered saline (PBS), containing 0.5% bovine serum albumin (BSA) was overlaid on the densified blood prior to centrifugation. The importance of the buffer layering on the buffy coat is demonstrated in the following assays.

Blood drawn into preservative tubes (WO 03018757) and stored at room temperature overnight was pooled and two 30 ml fractions were then transferred to 50 ml centrifuge tubes. Five ml iodixanol was added to the blood and the blood/ iodixanol mixture was mixed several times by inversion. The blood was then allowed to stand for five minutes. During this five minute waiting period, 500 µl of phosphate buffered saline (PBS), containing 0.5% bovine serum albumin (BSA), is gently overlaid on top of the blood in one of the 30 ml blood samples. The other had no buffer overlay. Both samples were then centrifuged at 800 xg for 20 minutes.

The samples are then placed in the LVSP apparatus for analysis. The plunger rate was set at 0.25 inches/minute to collect 8 mls of the buffy coat layer. Both the buffy coat fraction material in the 15 ml centrifuge tube and the buffy coat-depleted fraction in the 50 ml centrifuge tube are well mixed. 200 µl from each were removed and a complete blood count was performed on a Sysmex Hematology Analyzer. A total white blood cell count (WBC) was then determined for both fractions and expressed as a percent WBC recovery in the buffy coat.

The results are shown in Table 2. Also during collection of the sample lacking buffer overlay not all the buffy coat was collected due to cell adhesion to the sides of the 50 ml centrifuge tube wall, particularly as the fluid level dropped during sample acquisition. Thus, the buffer overlay is not only protecting the buffy coat from the liquid/air interface but is also preventing the cells in the buffy coat from adhering to the centrifuge tube wall. This dual protective action is continuous during the entire collection of the 8 ml buffy coat and associated material. As shown in Table 2, the

WBC from the buffy coat without a buffer overlay shows a significant loss in sample recovery.

Table 2

	WBC Recovery in Buffy Coat
Buffer Overlay	92%
No Buffer Overlay	67%

It is to be understood and appreciated that these examples are illustrative of the many potential applications of the instrument and method that may be envisioned by one of ordinary skill in the art, and thus are not in any way intended to be limiting of the scope of the invention. Accordingly, other objects and advantages of the invention will be apparent to those skilled in the art from the detailed description, together with the associated claims.

Claims

1. An automated method for the prepartion of large numbers of samples in rare cell analysis comprising:
    a. obtaining said sample containing said rare population of target cells;
    b. densifing said blood sample;
    c. adding a protective top layer;
    d. centrifuging densified blood sample in a capped centrigation vessel whereby said centrifugation results in a partioning with a top fraction containing white blood cells and said target cells;
    e. aspirating a volume from said top fraction from each sample whereby said rare cells remain morphologicaly and antigenically unaltered from a preparation state; and
    f. analyzing said rare cells.

2. The method of claim 1, whereby said aspiration comprises:
    a. replacing said vessel cap with a slideably sealed piston whereby said piston contains a head portion with an optimally shaped and positioned collection duct on a fluid surface to prevent loss of rare cells; and
    b. driving said piston into said vessel to impart an air space between plunger head and sample surface whereby said air space provides a uniform pressure on said sample surface.

3. The method of claim 1, whereby said densified blood is obtained with an addition of an agent from a group consisting of iodixanol, diatrizoic acid salts, and combination thereof.

4. The method of claim 1, whereby said densified blood is 1.09 g/ml.

5. The method of claim 2, whereby said piston is driven for an optimal time and distance inorder to separate said top fraction from said sample.

6. The method of claim 2, whereby a ratio of cross-sectional inner diameter of vessel to collection duct is approximately 1:10 to approximately 1:1.5.

7. The method of claim 2, whereby the ratio of cross-sectional inner diameter of vessel to collection duct is optimally in the range of approximately 1:5 to approximately 1:8.

8. The method of claim 1, whereby all components in contact with said sample are safely packaged for disposal within said slideably sealed piston.

9. The method of claim 1, whereby said volume is further immunomagnetically enriched prior to said analysis.

10. The method of claim 1, whereby said analyzing includes fluorescent imaging inorder to detect and enumerate said rare cells for clinical diagnostics.

11. The method of claim 1 whereby said analysis is from a group consisting of transcriptomic, proteomic, genomic, and combinations thereof.

12. An automated device for aspirating a densified blood sample comprising:
    a. a conical tube with cap whereby said conical tube contains at least 30 ml of centrifuged densified blood;
    b. a cap assembly containing plunger means and transfer tubing whereby said cap assembly readily dispenses and retracts said transfer tubing after replacement of said centrifuge cap;
    c. a plunger head forming a slideable seal with the inner wall of said tube;
    d. an air space between said plunger head and said sample whereby said air space provides a uniform pressure on said sample surface;
    e. a transfer tube optimally connected for fluid transfer from the fluid surface of the plunger head to a receiving vessel whereby said transfer tube forms a trumpet-shaped collection duct with a linear inner diameter gradient with said plunger head;

f. a processor system with bracket support for said tube, said cap assemble, transfer tube, and receiving vessel; and

g. a programmable electronic controller with a capability to store a protocol and execute said protocol via systemic control for components a through f.

13. The device of claim 12 whereby said blood contains a densifying agent from a group consisting of iodixanol, diatrizoic acid salts, and combinations thereof.

14. The device of claim 12 whereby said densified blood is partitioned between a density of 1.09 g/ml.

15. The device of claim 12 whereby said tube is a 50 ml polypropylene conical centrifuge sample tube.

16. The device of claim 12 whereby said transfer tube is positioned in the center of said plunger head.

17. The device of claim 12 whereby said transfer tube forms a trumpet-shaped collection duct with the open end in contact with the fluid sample.

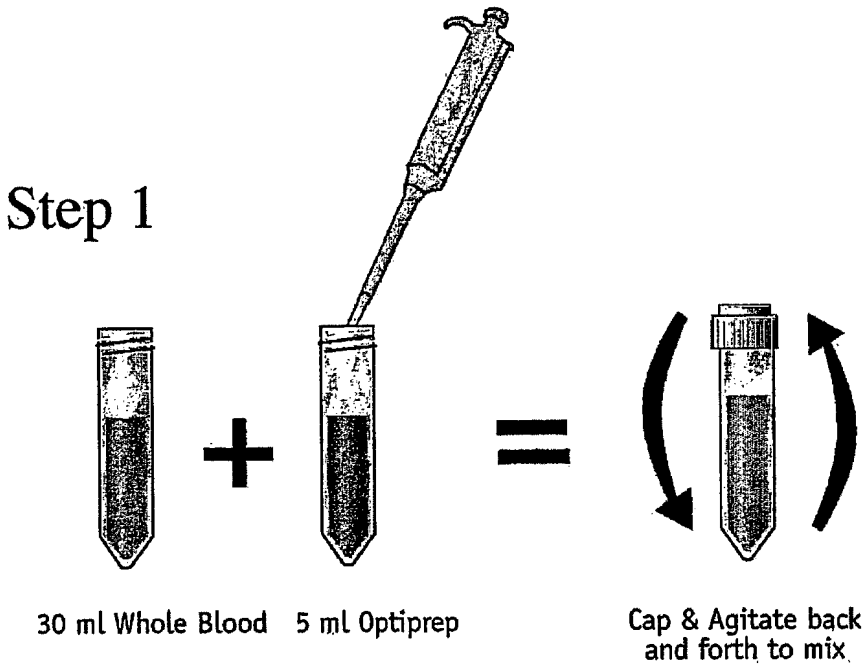
18. The device of claim 17 whereby said transfer tube forms a linear inner diameter gradient along said trumpet-shaped collection duct.

19. The device of claim 18 whereby a ratio of cross-sectional diameter of said transfer tube and said conical tube is in a range of approximately 1:10 to 1:1.5.

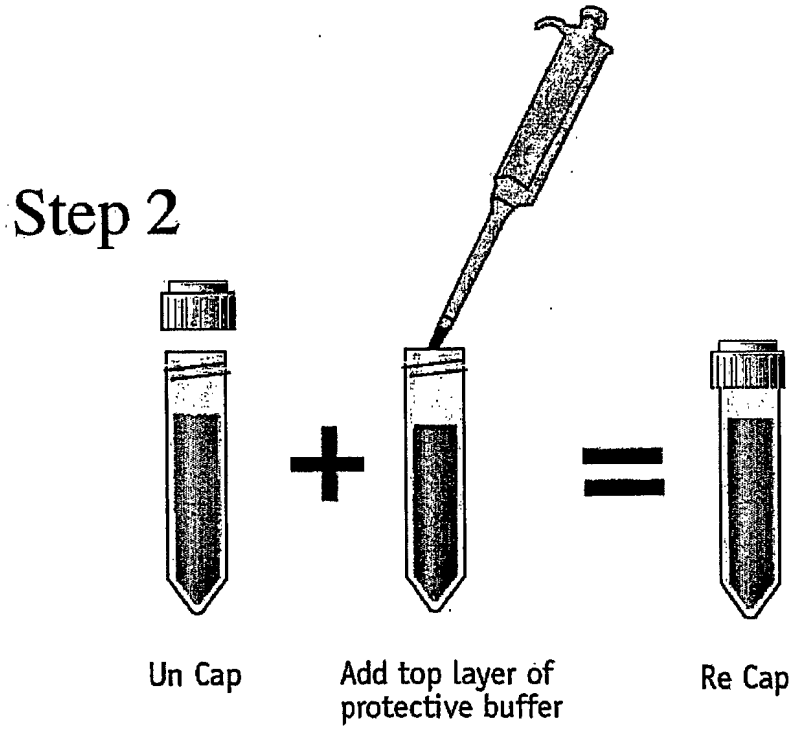
20. The device of claim 19 whereby a ratio of cross-sectional diameter of said transfer tube and said conical tube is preferably in a range of approximately 1:8 to 1:5.

21. The device of claim 19 whereby said receiving vessel contains a fluid level sensor.

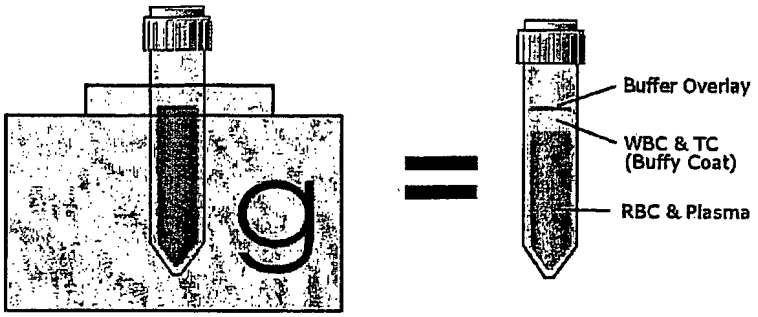
Figure 1


Step 1
30 ml Whole Blood
5 ml Optiprep
Cap & Agitate back and forth to mix

5


Step 2
Un Cap
Add top layer of protective buffer
Re Cap

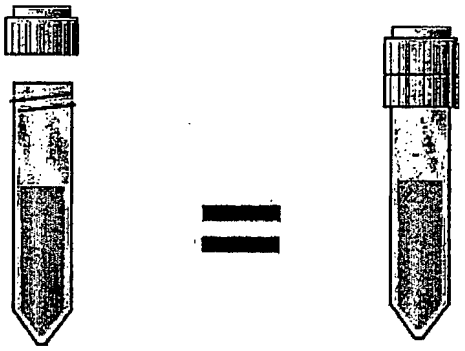
Step 3



Buffy Coat Separated
by centrifuge

5

Step 4



Remove and
discard Cap

Re-Seal with Immunicon
Plunger Cap

Figure 2

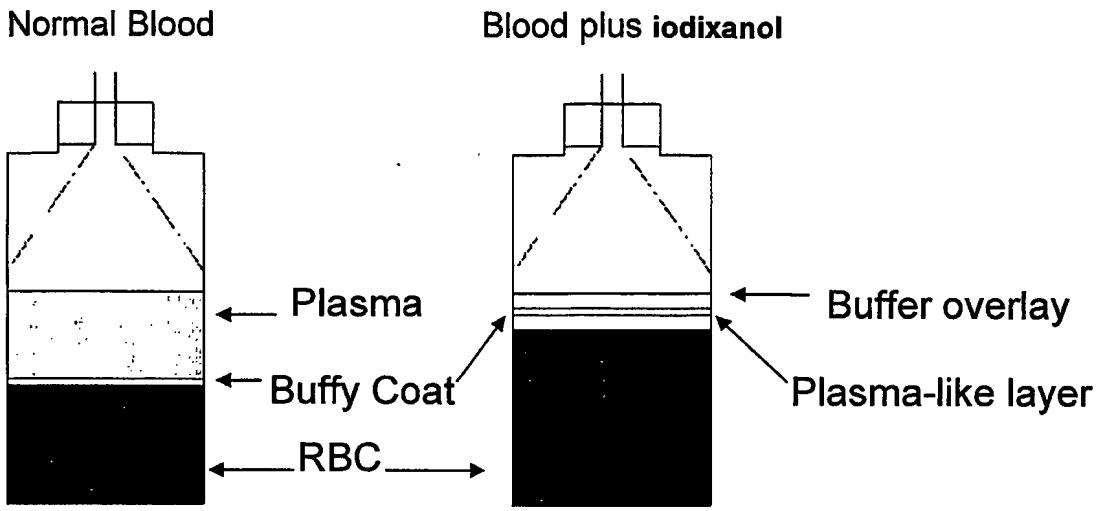
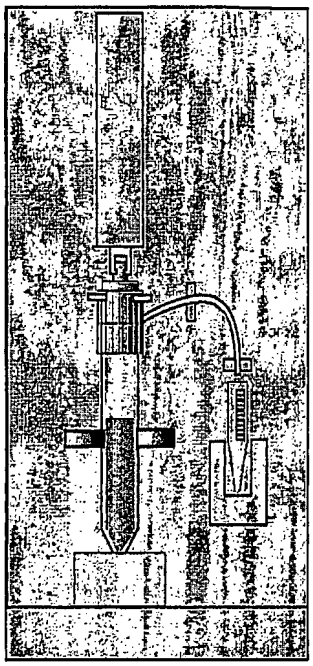
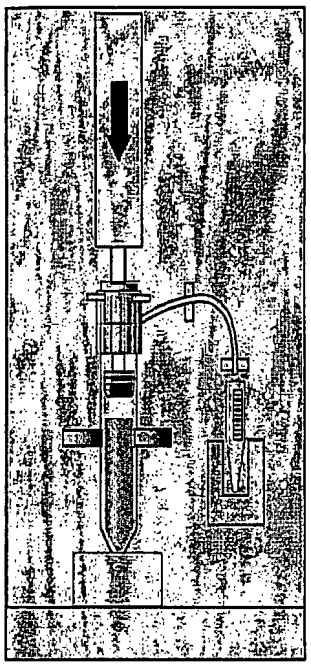

Normal Blood
Blood plus **iodixanol**
Plasma
Buffy Coat
RBC
Buffer overlay
Plasma-like layer

Figure 3

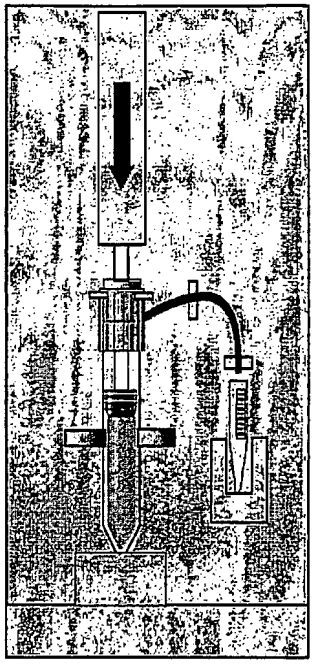


User loads:
- Spun 50ml tube w/tube cap
- Empty 7.5 Sample vial

Places transfer tube in position

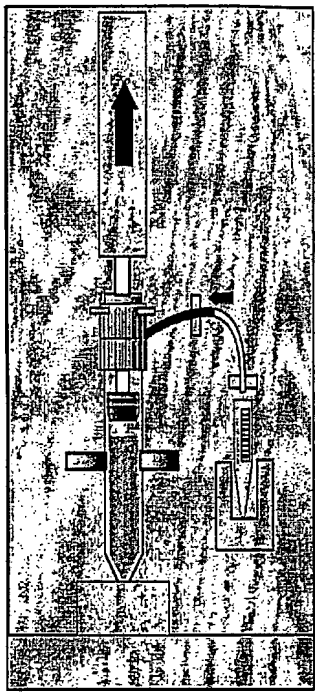


Lower plunger tube into 50ml tube

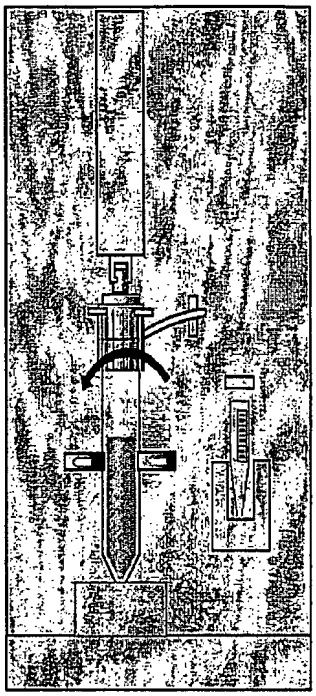


Stop plunger tube when sample vial has 7.5ml buffy coat

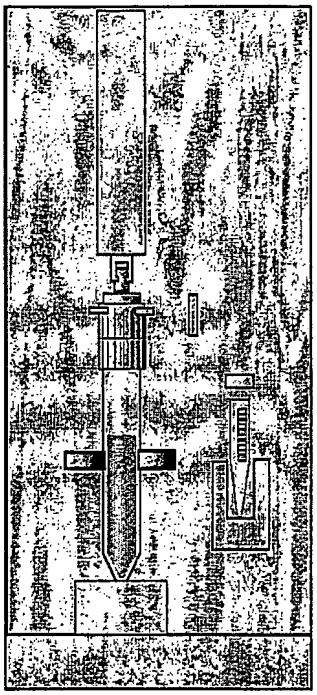
Figure 4



Plunger rises, drawing all remaining bio hazard out of tube by reverse suction



Plunger in fully raised position, 50ml Tube spins wrapping pigtail tube inside cap



User removes and disposes 50ml tube and cap assembly.

User takes 7.5ml buffy coat sample to Autoprep System.

Figure 5

5

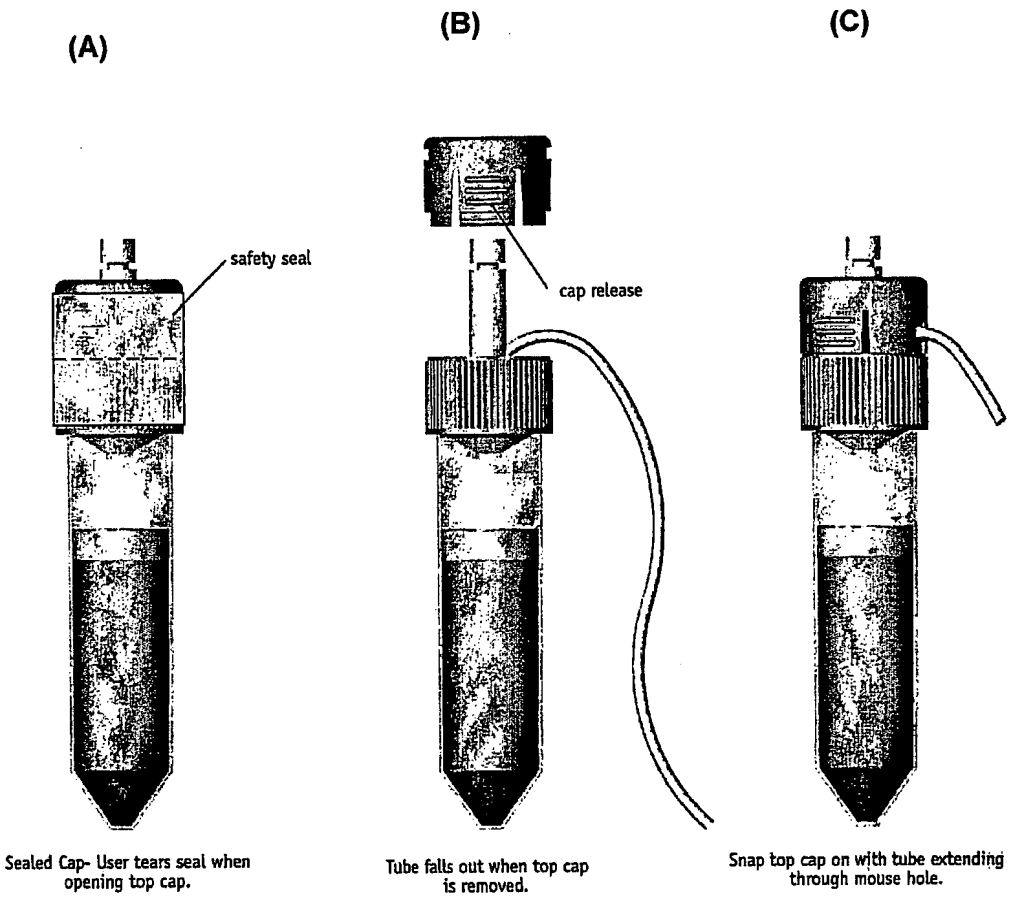

(A)
(B)
(C)
safety seal
cap release
Sealed Cap- User tears seal when opening top cap.
Tube falls out when top cap is removed.
Snap top cap on with tube extending through mouse hole.

Figure 6

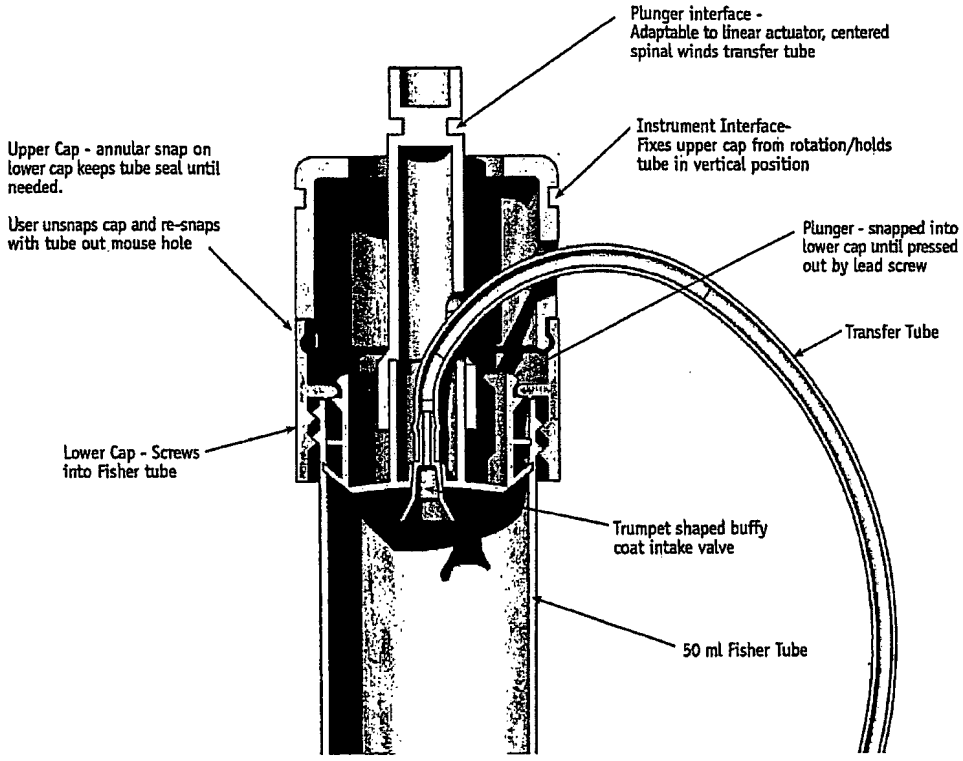

Plunger interface -
Adaptable to linear actuator, centered
spinal winds transfer tube
Instrument Interface-
Fixes upper cap from rotation/holds
tube in vertical position
Upper Cap - annular snap on
lower cap keeps tube seal until
needed.
User unsnaps cap and re-snaps
with tube out mouse hole
Plunger - snapped into
lower cap until pressed
out by lead screw
Transfer Tube
Lower Cap - Screws
into Fisher tube
Trumpet shaped buffy
coat intake valve
50 ml Fisher Tube

Figure 7

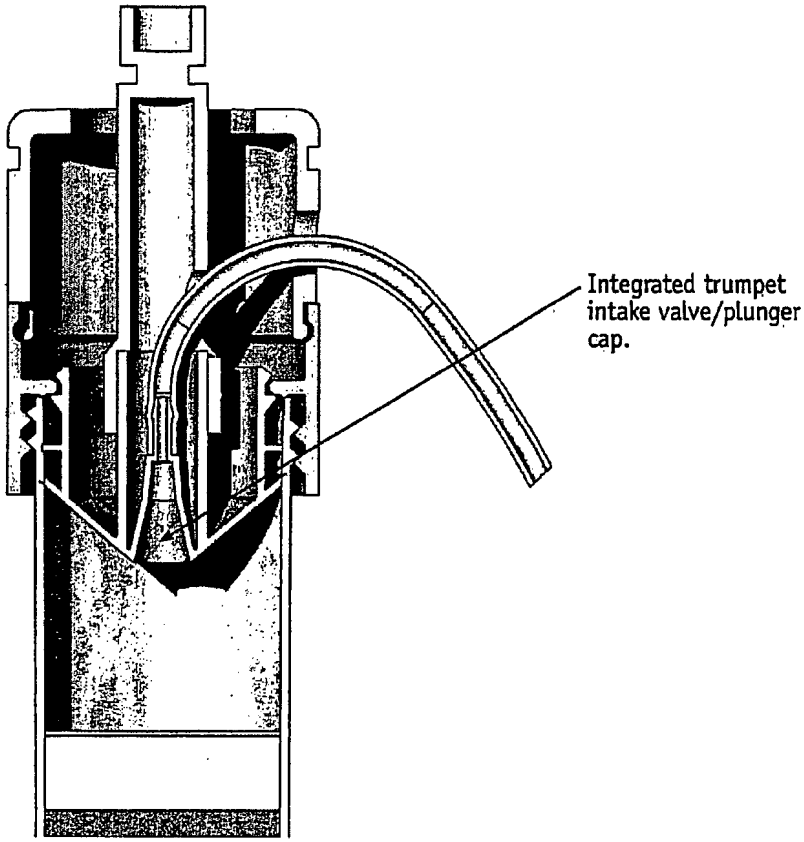
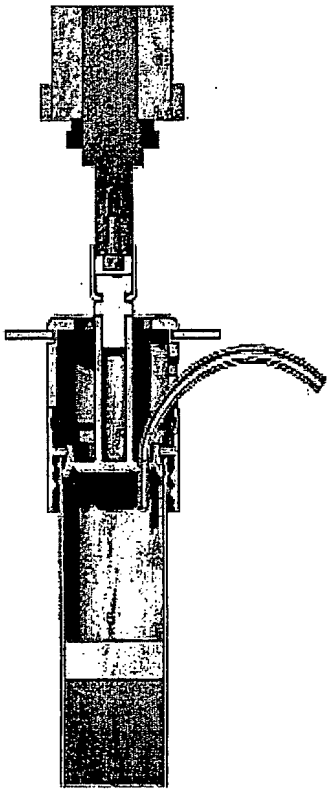


Figure 8

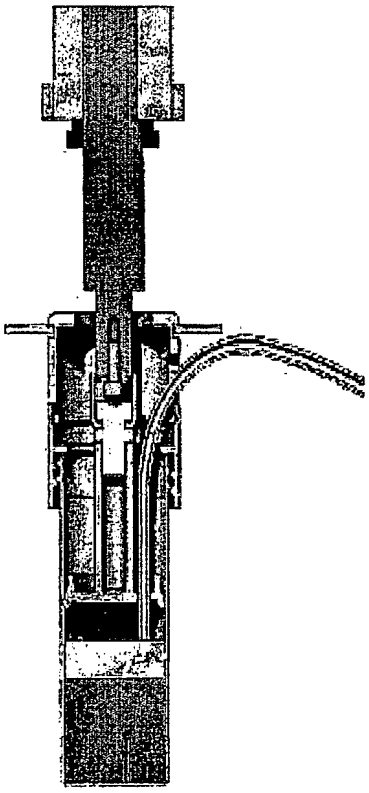
5

(A)



Cross section - plunger home position

(B)



Cross section - extended position

Figure 9

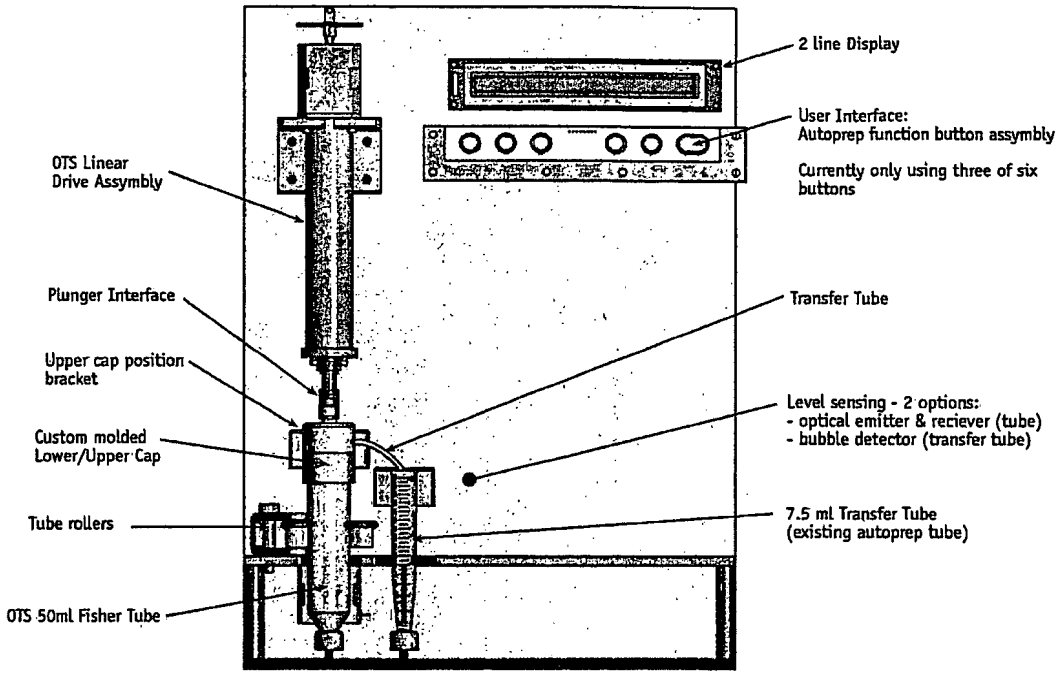
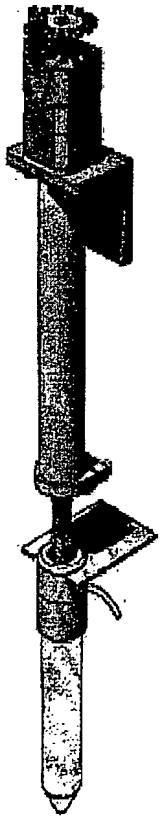
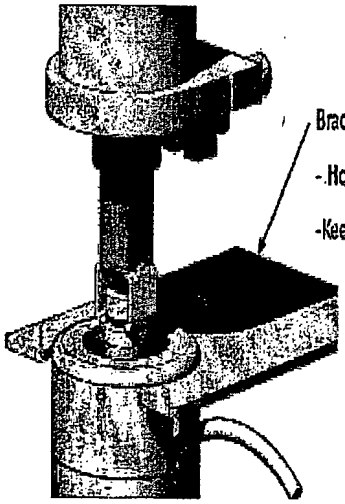

2 line Display
User Interface:
Autoprep function button assymbly
Currently only using three of six buttons
OTS Linear Drive Assymbly
Plunger Interface
Upper cap position bracket
Custom molded Lower/Upper Cap
Tube rollers
OTS 50ml Fisher Tube
Transfer Tube
Level sensing - 2 options:
- optical emitter & reciever (tube)
- bubble detector (transfer tube)
7.5 ml Transfer Tube
(existing autoprep tube)

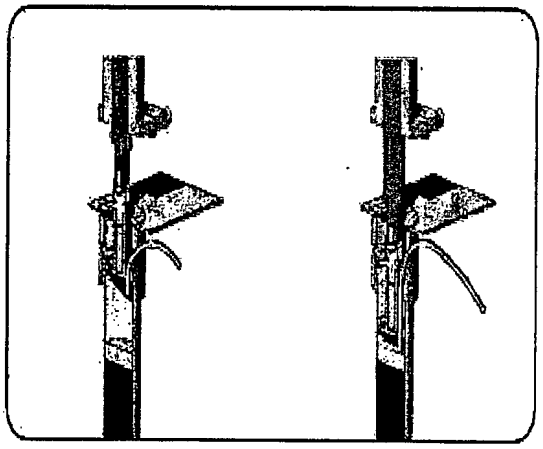
Figure 10



Tube location relative to lead screw

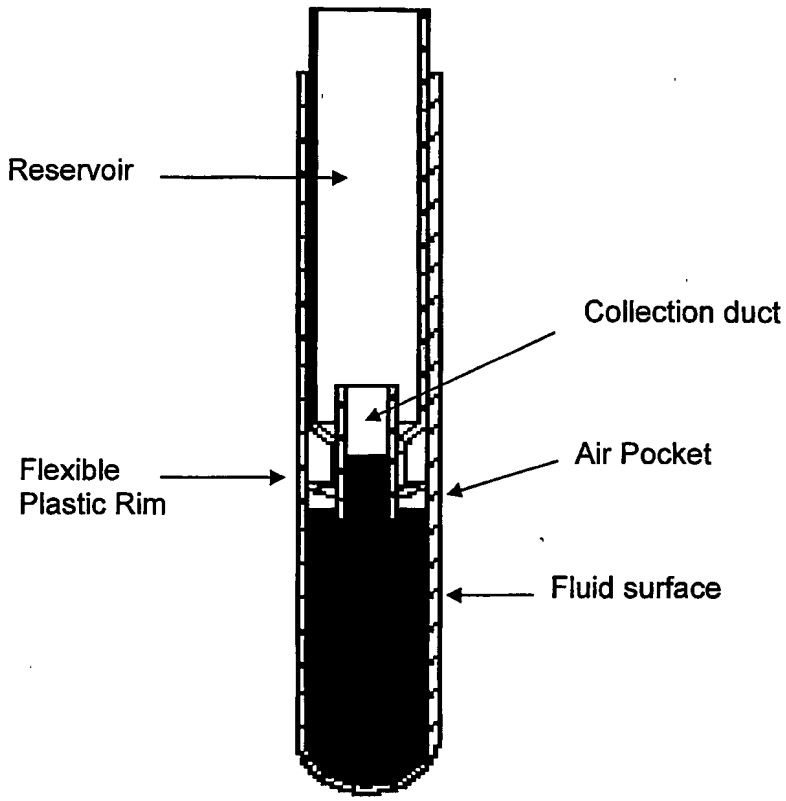


Detail of attachement concept



Cross section - plunger travel

Figure 11 Plunger-reservoir Device



5

Figure 12 WBC Recovery with an off-center on-center and flush on-center